EXPERIMENT 2: Polymer Synthesis after Infection

Bacteria as noted in Experiment 1.

Bacteriophage as noted in Experiment 1.

Media

For MS2, TPG medium:

KCl	0.11 M	KH_2PO_4	1.0 mM
NaCl	0.008 M	Na pyruvate	0.073 M
NH_4Cl	0.05 M	$FeCl_3$	2 μμM
Tris	0.10 M	$CaCl_2$	0.1 mM
Bring to pH 7.4 with HCl		$MgCl_2$	1.0 mM
Na_2SO_4	0.16 mM	Glucose	0.2 gm/L

For T4, M-9 medium:

NH_4Cl	1.0 gm
$MgSO_4$	0.13 gm
KH_2PO_4	3.0 gm
Na_2HPO_4	6.0 gm
Glucose	4.0 gm
Water	1 liter

For T4 infection, add L-tryptophan, 20 mg.

The purpose of this experiment is to measure nucleic acid and protein synthesis in cells infected with normal T4 or MS2 and amber mutants of these phages. In the case of MS2, E. coli will be treated with actinomycin D prior to infection in order to inhibit host RNA and protein synthesis. Each pair of students will use a specific phage as indicated below:

A. T4 series

1. Uninfected B
2. T4r in B
3. T4 am122 in B
4. T4 am102 in B
5. T4 amB17 in B
6. T4r in B

B. MS2 series

1. MS2 in C3000
2. Uninfected C3000
3. MS2 am2 in C3000
4. MS2 am9 in C3000
5. MS2 ts4 in C3000
6. MS2 in C3000

To be supplied:

1. B in M-9 medium at 2 x 10^9/ml
2. C3000 in Tris HCl at 4 x 10^9/ml
3. Phage at 10^{10} pfu/ml (T4) or 4 x 10^{10}/ml (MS2)
4. 13 x 100 mm tubes containing H^3-thymidine (T), H^3-uridine (U) or C^{14}-valine (A)
5. Filter paper discs, pins and foam rubber pin cushion
6. TPG-actinomycin (5.5 μg/ml actinomycin)
7. Thymidine-uridine-amino acid mixture (TUA mix)
8. EDTA-Tris
9. 5% TCA
10. 10% TCA
11. Alcohol-ether (1:1 by volume)
12. Ether
13. Scintillation counter vials
14. 100 μL disposable pipettes

Before the experiment begins, prepare filter paper discs for samples as demonstrated.

Protocol for T4 -- Period 1

-2' - Pipette 0.2 ml of B into a 13 x 100 mm tube in 30° water bath.
0' - Add 0.2 ml of phage (2 x 10^9 pfu) or in case of uninfected control, 0.2 ml of nutrient broth.
4' - Add 2.0 ml of M-9 medium
5' - Distribute 0.6 ml to each isotope tube at 30° (T, U, Ⓐ). Leave the remaining culture at 30° (Tube 4). Shake all tubes.
7' - Remove 0.1 ml from each isotope tube (T, U, Ⓐ) with a separate capillary pipette and deliver onto filter paper discs marked T7, U7, Ⓐ 7, respectively. (Remember the sample is radioactive!) Immediately after applying sample to disc, add 1 drop "TUA mix" to quench (use a Pasteur pipette). (Keep capillary pipettes in empty 13 x 100 mm tubes for further use. Do not mix up T, U, A pipettes.)
10' - Dilute 0.1 ml from tube 4 to 10^{-3} and 10^{-4} and spread 0.1 ml on T4 plates.
15')
30')- Repeat sampling as at 7'
60')
Leave all tubes in bath. Store discs in your locker.

Protocol for MS2 -- Period 2

-10'- Heat 0.2 ml EDTA-Tris to 41° in a can of tap water.
-8' - Add 0.2 ml C3000 and keep at 41°
-5' - Add 0.2 ml of EDTA-treated cells to 2 ml TPG-actinomycin, which has been shaking at 37° in a 18 x 150 mm tube.
0' - Infect cells at multiplicity of 5 (total of 2 x 10^9 pfu)
Controls: do not add phage

5' - Distribute 0.6 ml of cells to each of two tubes labelled Ur (for H^3-uridine) and A (for C^{14}-amino acids).

10' - Withdraw 100 µl from Ur and from A with capillary pipettes and apply to Ur-10 and A-10 discs, respectively. Immediately add 1 drop of TUA mix to each disc with a Pasteur pipette. Keep pipettes in clean tubes (Ur or A) for further use.

20')
30')- Repeat sampling
60')

Leave all tubes in water bath! Store discs in your locker.

Washing filter paper discs -- Period 3

Put all T, U, Ur discs in one 250 ml beaker, leaving pins in the discs. Do not touch discs with fingers; handle the pins only. This is beaker 1.

Put all the A and (A) discs in a second 250 ml beaker. This is beaker 2.

Label each beaker with your pair number.

Keep beaker 1 in an ice bath, add ice-cold 5% trichloracetic acid (TCA) to cover discs, and allow to soak for 10 minutes. Decant the TCA carefully (radioactive!) and repeat 4 more times for 10' periods. Wash discs twice with alcohol-ether for 5 minutes, twice with ether, and allow to dry.

To beaker 2 add cold 10% TCA and allow discs to soak for 30 minutes. Decant carefully (radioactive!) and add 5% TCA. Heat at 90° in a pan of water for 15 minutes. Wash the discs three times with 5% TCA for 5 minutes at room temperature and then with alcohol-ether and ether as above.

Place discs without pins in scintillation vials using gloved hand, and label tops with pair number and disc number. Instructors will collect and count your vials in a liquid scintillation counter. When the vials are counted, the results will be returned to you. Plot your results and compare them with those of others.